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Light and Electron Microscopic Study of Primate (Macaca Mulatta) Liver After Large Doses of High-Energy (2.3 Bev) Protons

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Several investigators have dealt with the ultrastructural sequelae in liver following whole-body gamma and x-irradiation.[1,2,3,4] A study of the effects of particulate radiation on liver was reported from this laboratory;[5] the primates were exposed to 32 Mev protons with whole-body exposure technique but the limited penetration of 32 Mev protons resulted in a partial-body dose distribution. This communication reports the sequential morphologic events in liver following whole-body exposure of primates to high-energy protons which penetrated the whole animal.

METHODS

Seven primates (Macaca mulatta) were irradiated with 2.3 Bev protons from the Cosmotron at the Brookhaven National Laboratory. Two sham irradiates served as controls. The total-body doses of 3,000 rads were estimated from measurements made at the surfaces of the animals. The animals weighed approximately 5 Kg each. Details of dose calculation and estimation and of animal exposure have been previously reported.[6] Both experimental and the sham -irradiated control animals were kept in the exposure cylinder for the same length of time. All animals were restricted to a diet of water for 24 hours prior to irradiation and fed

standard monkey chow, fruit, and water ad libitum following exposure.

The animals were killed at 15 minutes, 6, 12, 18, 24, 36, and 48 hours after completion of irradiation. Tissue for paraffin embedding was fixed in neutral-buffered 10% formalin; tissue for electron microscopy was cut into 1 mm cubes and fixed either in cacodylate-buffered (pH 7.4) osmium tetroxide alone for 2 hours at 4°C or in 5% cacodylate-buffered (pH 7.4) glutaraldehyde followed by a 2-hour post-fixation in buffered osmium tetroxide also at 4°C.[7] The tissue blocks were rapidly dehydrated in graded ethanols and then infiltrated with and embedded in a mixture of Epon and Araldite.[8] The embedments were polymerized overnight at 80°C. One-micron plastic sections prepared for light microscopy were stained with a modified Methylene blue-Azure II procedure[9] which was counterstained with basic fuchsin. Silver and gray ultrathin sections prepared for electron microscopy from the same blocks were stained first with 0.5% uranyl acetate[10] for 15 to 30 seconds and then with 0.1% lead citrate[11] for 30 to 60 seconds. The material was studied and photographed with RCA (model EMU-3H) and Siemens (model IA) electron microscopes at 100 and 80 Kv respectively.

RESULTS

15 minutes:

The overall histologic appearance was unremarkable (Fig. 1). Dark cells were noted as infrequently as in our control material. Small numbers of cytoplasmic lipid droplets were present in some hepatocytes.

Cytologically, the hepatocytic ultrastructure varied little from that of our control tissue. Club-like swellings of microvilli along the canaliculi which we have not encountered in control Rhesus livers were occasionally seen (Fig. 2). Small cytoplasmic clefts similar to those previously reported in irradiated liver[5] were present (Fig. 3).

6 hours:

The accumulation of large amounts of cytoplasmic lipid throughout the hepatic lobule was readily apparent with the light microscope as well as with the electron microscope (Fig. 4). The spongy appearance of hepatocytic cytoplasm appeared, electron microscopically, to correlate with aggregates of dilated smooth endoplasmic reticulum and vesicles which were randomly scattered throughout the cytoplasm (Fig. 5). Similar but smaller focal aggregates were noted rarely in control liver cells. Light gray amorphous material frequently filled distended spaces of Disse (Fig. 6).

12 hours:

Cytoplasmic lipid droplets within hepatocytes were more numerous than in the previous specimens (Fig. 7). Frequently glycogen granules were loosely arranged; islands of smooth reticulum, devoid of recognizable glycogen, were common. Small cytoplasmic clefts were still present. Cytoplasmic blebbing was encountered frequently along canaliculi and spaces of Disse.

The sinusoidal lining cells (Kupffer cells) were enlarged and prominent. A wide variety of cytoplasmic inclusions was noted; unidentified material was occasionally arranged in crystalline array (Fig. 8).

18 hours:

The histologic appearance of this specimen differed from the previous specimens in two respects. The hepatocytes were heavily laden with cytoplasmic lipid and dark cells were very numerous (Fig. 9). Although many extremely dense, shrunken hepatocytes were present, a broad spectrum of various degrees of shrunkenness involved more than 25% of the hepatocytes.

Some sinusoids contained small thrombi (Fig. 10); the distribution of these thrombi was not obviously correlated with the distribution or severity of hepatocytic

alteration judged by either light or electron microscopy. Electron-dense material was frequently located in the space immediately surrounding hepatocytes (Fig. 11). Material of similar density was also noted within profiles of endoplasmic reticulum.

Many hepatocytes contained large, dense granules, some of which contained recognizable cytoplasmic material. These cytolysosomes were present in cells that were otherwise normal and in cells that were generally degenerating (Fig. 12). One hepatocyte with normal cytoplasmic ultrastructure had a swollen nucleus with condensed, chromatin masses (Fig. 13).

24 hours:

Fewer dark cells were present at this point and, although much cytoplasmic lipid in the form of large droplets was present, the overall histologic pattern was well preserved (Fig. 14). Electron microscopically, there was little variation of ultrastructure between cells. The cytolysosomes present in the 18-hour specimen were not present in this specimen. Cytoplasmic clefts and foci of dilated smooth vesicles were still present.

36 to 48 hour :

Light microscopically, partitioning of the cytoplasmic contents, as in control tissue, was clearly evident; islands of

non-granular cytoplasm usually correlated with ultrastructural zones of smooth endoplasmic reticulum were set off against the granular, organelle-filled cytoplasm (Fig. 15 & 16). Most lipid droplets were present in such areas. The lipid content was decreased in the 36-hour specimen and markedly decreased by 48 hours. Cytolysosomes were not present in hepatocytes; Kupffer cells still contained many dense bodies; small numbers of clefts remained.

DISCUSSION

Dosimetry of high-energy, particulate irradiation is problematical at best. We have not been concerned with this aspect of the study for this communication and have merely presented the morphologic data in irradiated primate liver that was sampled at various time intervals following whole-body exposure.

A striking finding was the progressive increase in hepatic lipid which was first noted at 15 minutes and persisted until 24 hours, after which it leveled off and then decreased sharply. At 24 hours the myriads of small droplets were lacking; at 48 hours the only lipid seen light microscopically was in the form of isolated large droplets present in some cells. Glycogen granules with

recognizable ultrastructure progressively decreased in number and concentration.

Small cytoplasmic clefts, were present in large numbers in the experimental livers. The fine structure of the cleft was similar to that of those previously reported in primate liver following 32 Mev proton irradiation.[5] Similar clefts have also been seen in humans with phenylketonuria and biliary atresia.[12]

The number of dark cells in the post-irradiation tissue reached a maximum at 18 hours and fell back within normal limits at 36 and 48 hours. This increased incidence of dark cells paralleled the incidence of cells showing obvious signs of degeneration at the level of fine structure such as clumping of nuclear chromatin and cytolysosome formation.

Distinctive mitochondrial alterations, especially of the type encountered in livers exposed to 32 Mev protons, were not present in this material.[5] Deviations from the normal size and shape of liver mitochondria seemed to be present but statistically meaningful data is not available and we did not have knowledge of the lobular zone at all times.

The morphologic data in this series of irradiated primates followed a sequence which was similar to that

described for dog pancreas although the time scale and type of radiation were different.[13,14] A certain proportion of the cells manifested signs of immediate damage; some died; others survived the insult, apparently ridding themselves of damaged organelles, and returned to normal structure and, perhaps, to normal function.

FIGURES

The light micrographs are all of one-micron sections of Epon-Araldite-embedded, osmicated tissue stained with Methylene blue-Azure II and basic fuchsin.

Figure 1 Light micrograph of liver at 15 minutes post-irradiation. There is a slight increase of minute cytoplasmic lipid droplets. x 500.

Figure 2 Microvillar "blebs" (B) of an hepatocyte protruding through the space of Disse and extending into the sinusoid. 15 minutes post-irradiation. x 36,000.

Figure 3 Hepatocyte containing several cytoplasmic "clefts" (arrows). Occasional large and irregular mitochondria are present in this field (A & B). 15 minutes post-irradiation. x 20,400.

Figure 4 Light micrograph of liver at 6 hours post-irradiation. Abundant cytoplasmic lipid is present in form of large and small droplets. x 500.

Figure 5 Hepatocytes adjacent to a bile canaliculus (C). Aggregates of dilated vesicles (*) are present in both cells. 6 hours post-irradiation. x 25,000.

Figure 6 Enlarged space of Disse (D) between hepatocytes (H) and sinusoidal lining cells (K). In addition to unit collagen fibers and fine filamentous material, the space contains a gray amorphous material. 6 hours post-irradiation. x 25,000.

Figure 7 Light micrograph of liver at 12 hours post-irradiation. There is a prepondenance of lipid in the form of large cytoplasmic droplets. x 500.

Figure 8 Sinusoidal lining cell (Kupffer cell) containing cytoplasmic crystalline array in a membrane-bound space. 12 hours post-irradiation. x 32,000.
INSET: Membrane-bound crystalline material. x 65,000.

Figure 9 Light micrograph of liver at 18 hours post-irradiation. Cytoplasmic lipid is present. There is a remarkable increase in the number of dark cells. x 500.

Figure 10 Sinusoidal area containing a thrombus. Fibrin, platelets, and erythrocytes are present. 18 hours post-irradiation. x 11,500.

Figure 11 Intercellular space (ICS) between hepatocytes. An irregularly dense material fills the space which widely separates two hepatocytes. 18 hours post -irradiation. x 9,000.

Figure 12 Cell containing multiple lysosomal bodies. The identity of this cell type is not clear; it may represent a distorted hepatocyte containing cytolysosomes or a phagocyte containing phagosomes. 18 hours post-irradiation. x 10,400.

Figure 13 Hepatocyte containing a distorted nucleus. The lumen of the nuclear envelope is distended. The chromatin is aggregated in an unusual fashion (not typical of early mitosis) and is separated by a finely granular material. The cytoplasm looks intact. 18 hours post-irradiation. x 17,900.

Figure 14 Light micrograph of liver at 24 hours. Large lipid droplets are numerous. A broad spectrum of dark cell intensities is present in this section. x 500.

Figure 15 Light micrograph of liver at 48 hours. Relatively few large lipid droplets remain. Only a few very dark cells are present. The majority of hepatocytes appear to be histologically normal. x 500.

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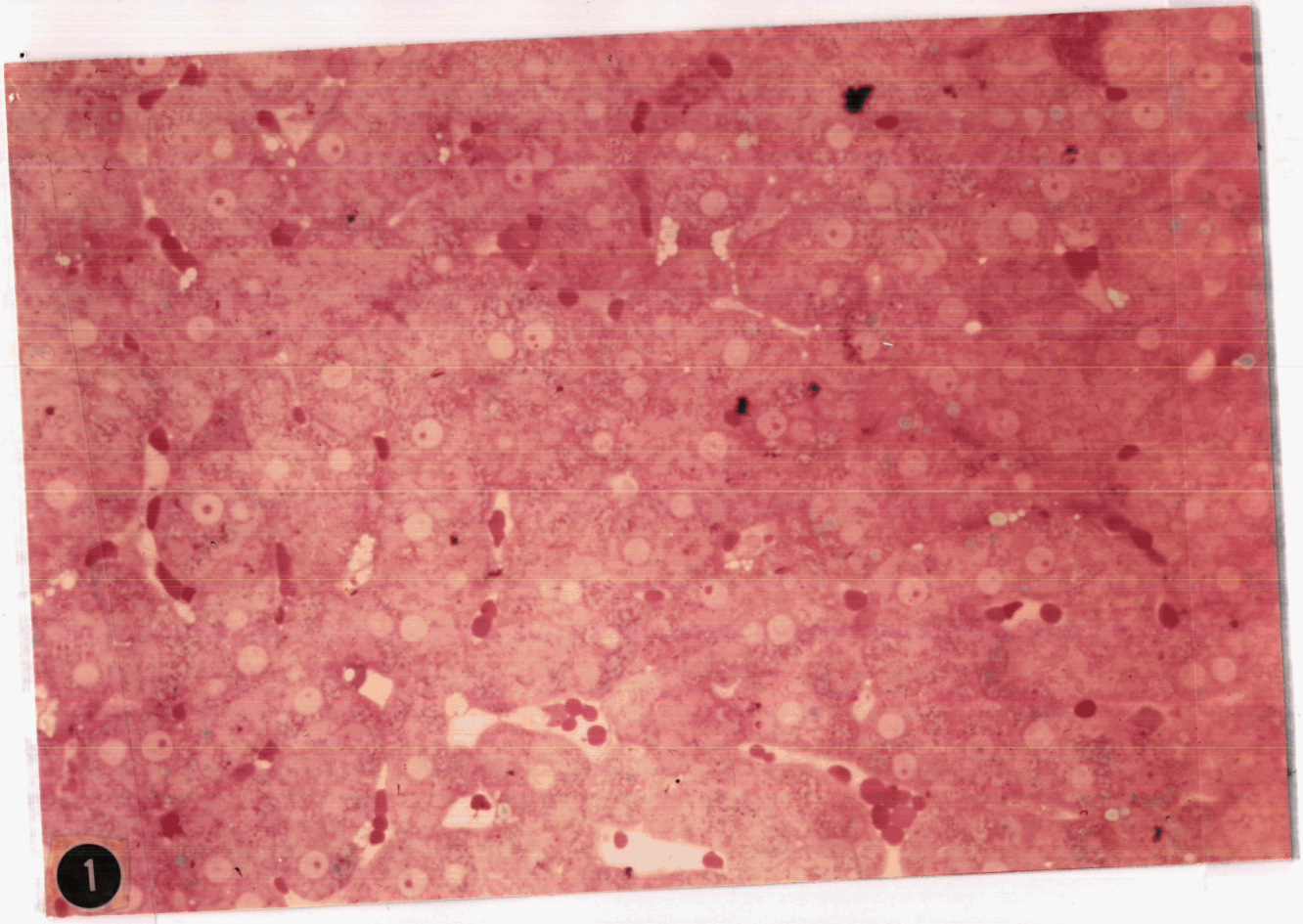

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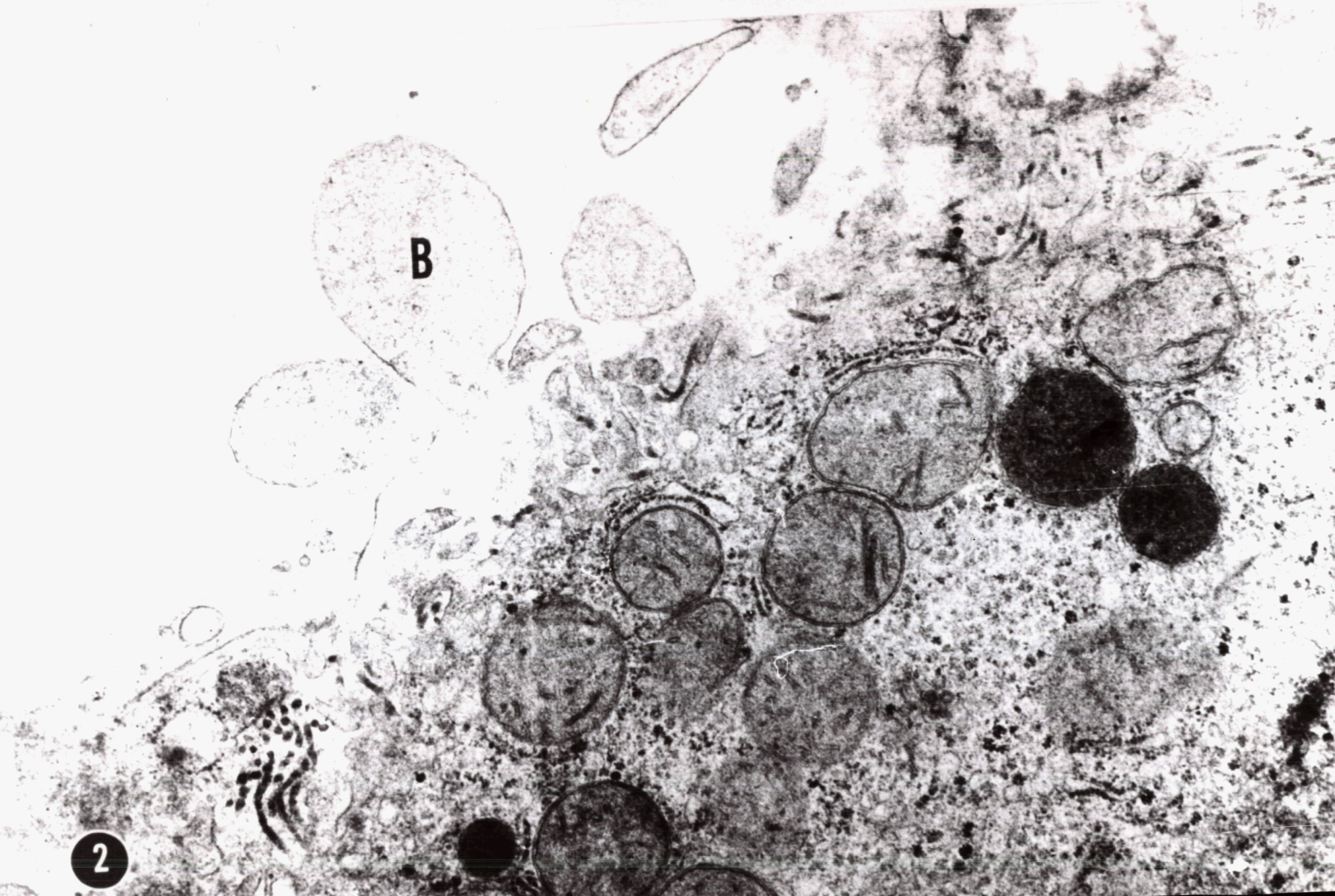

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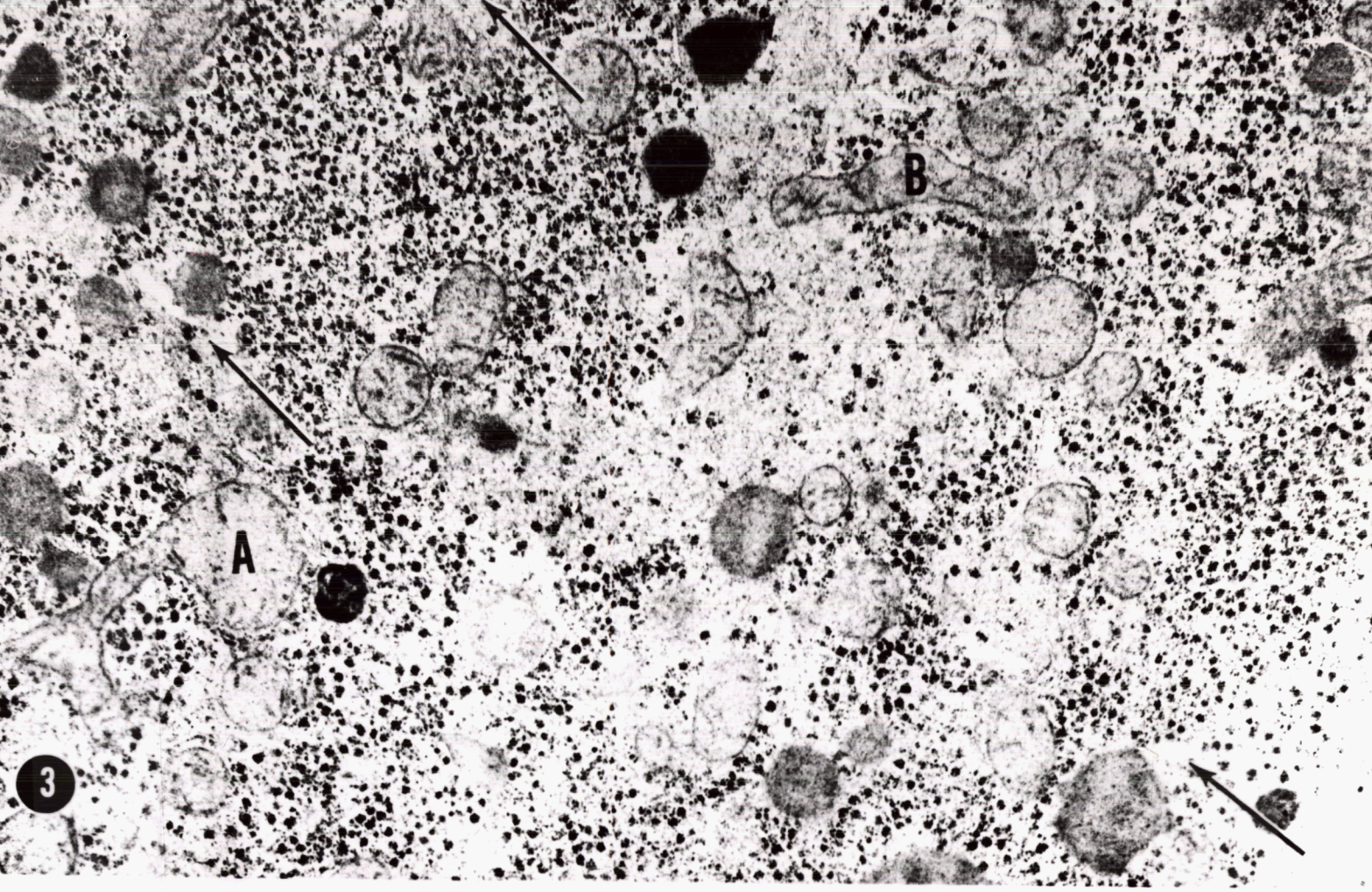

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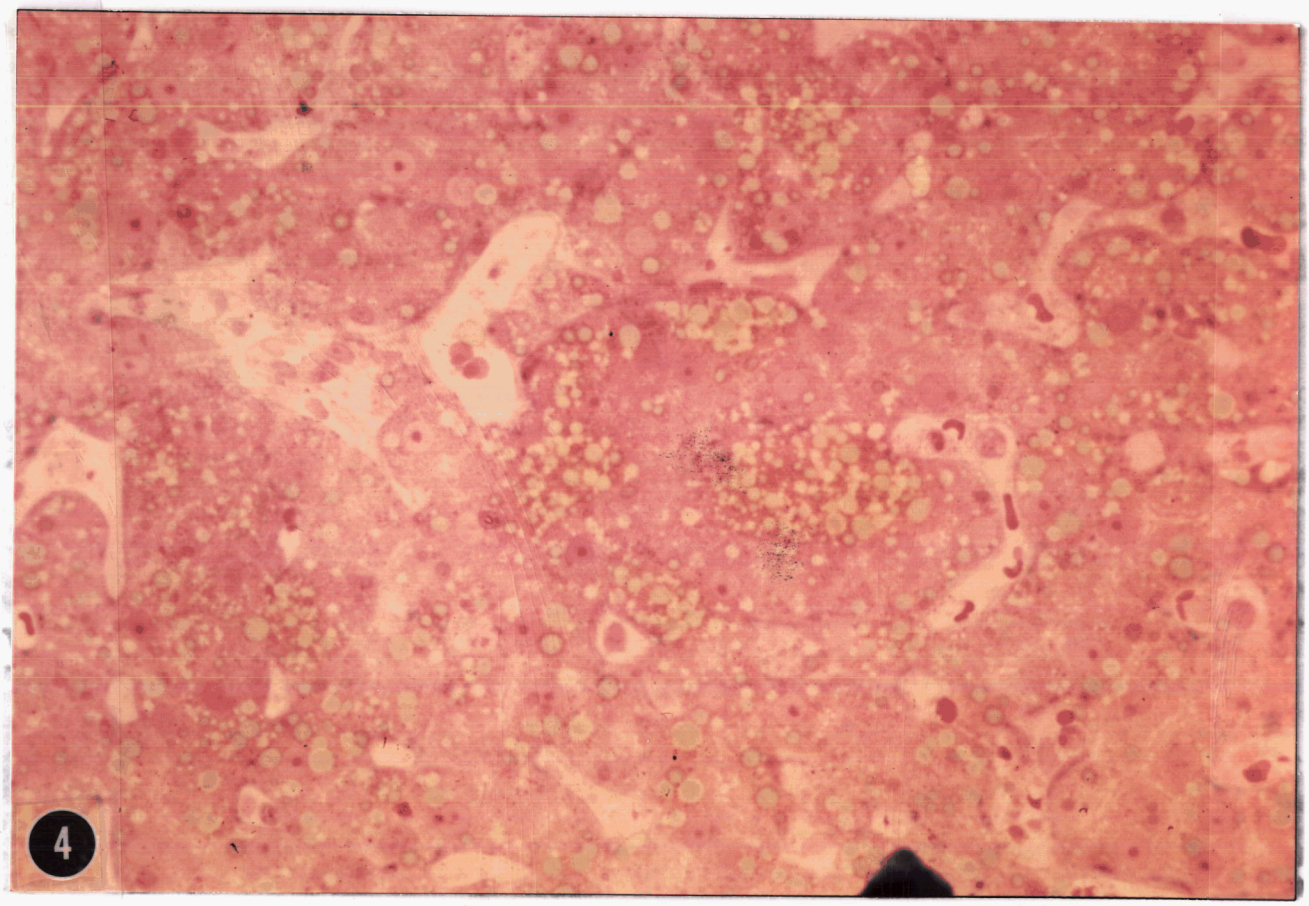

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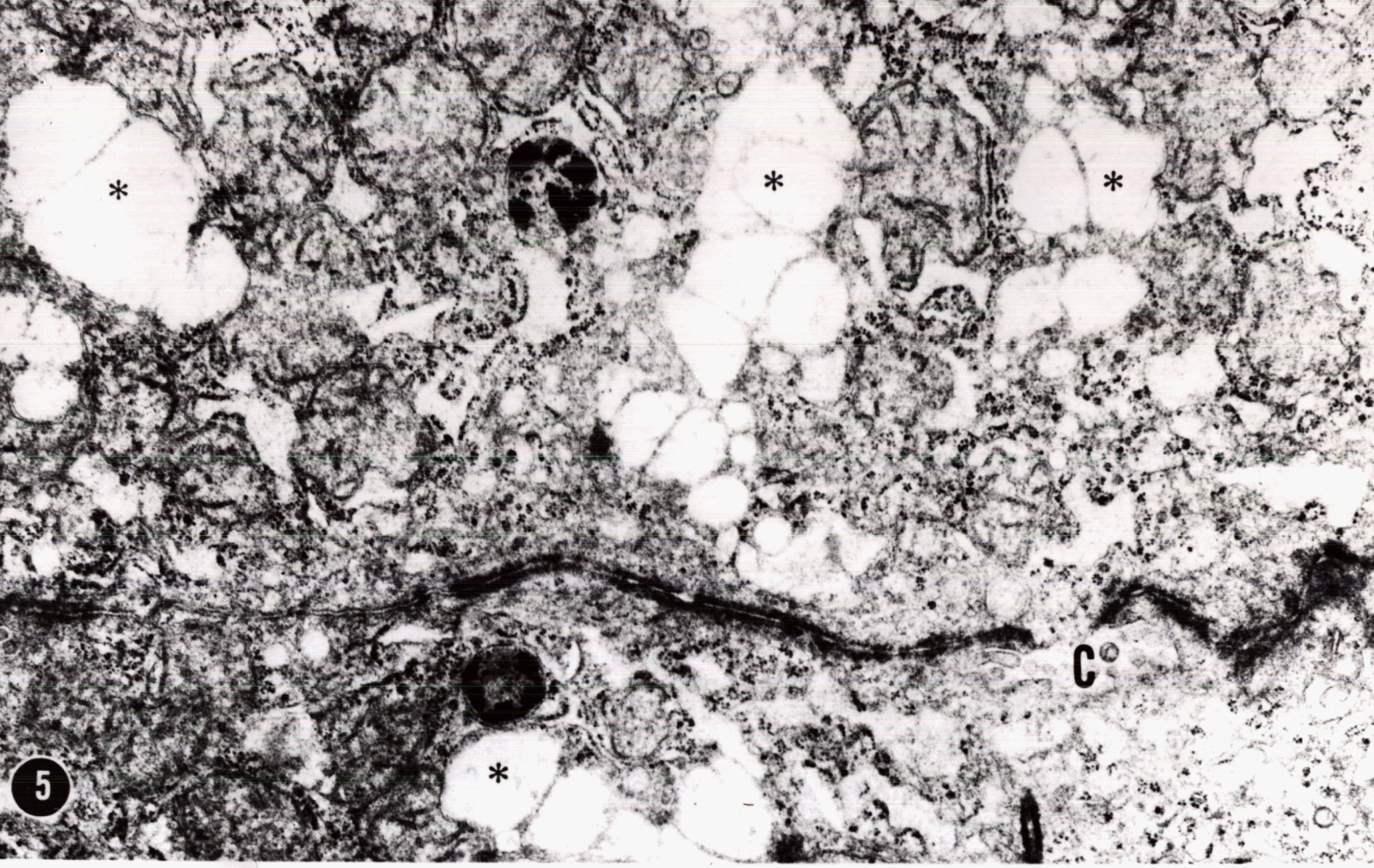
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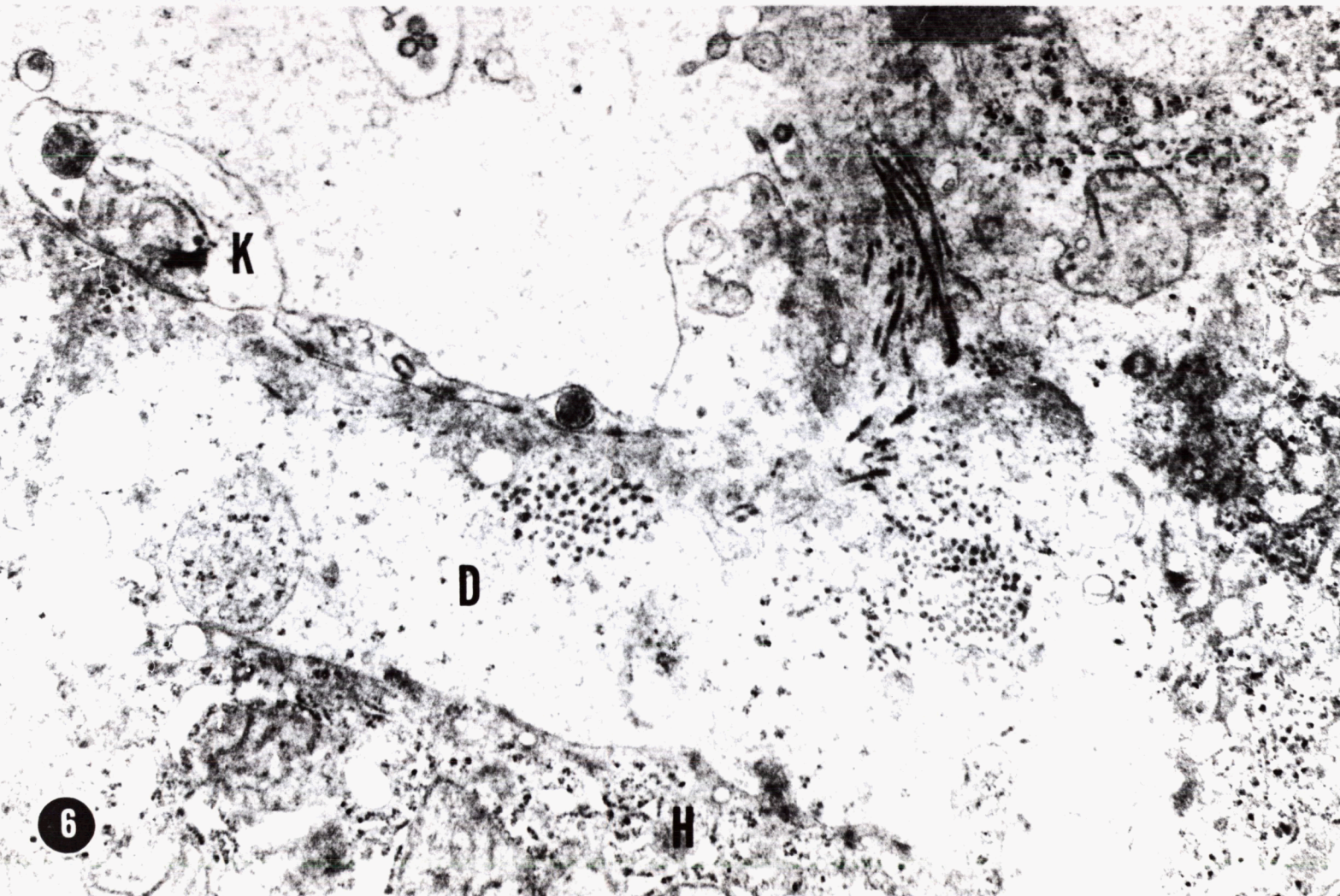
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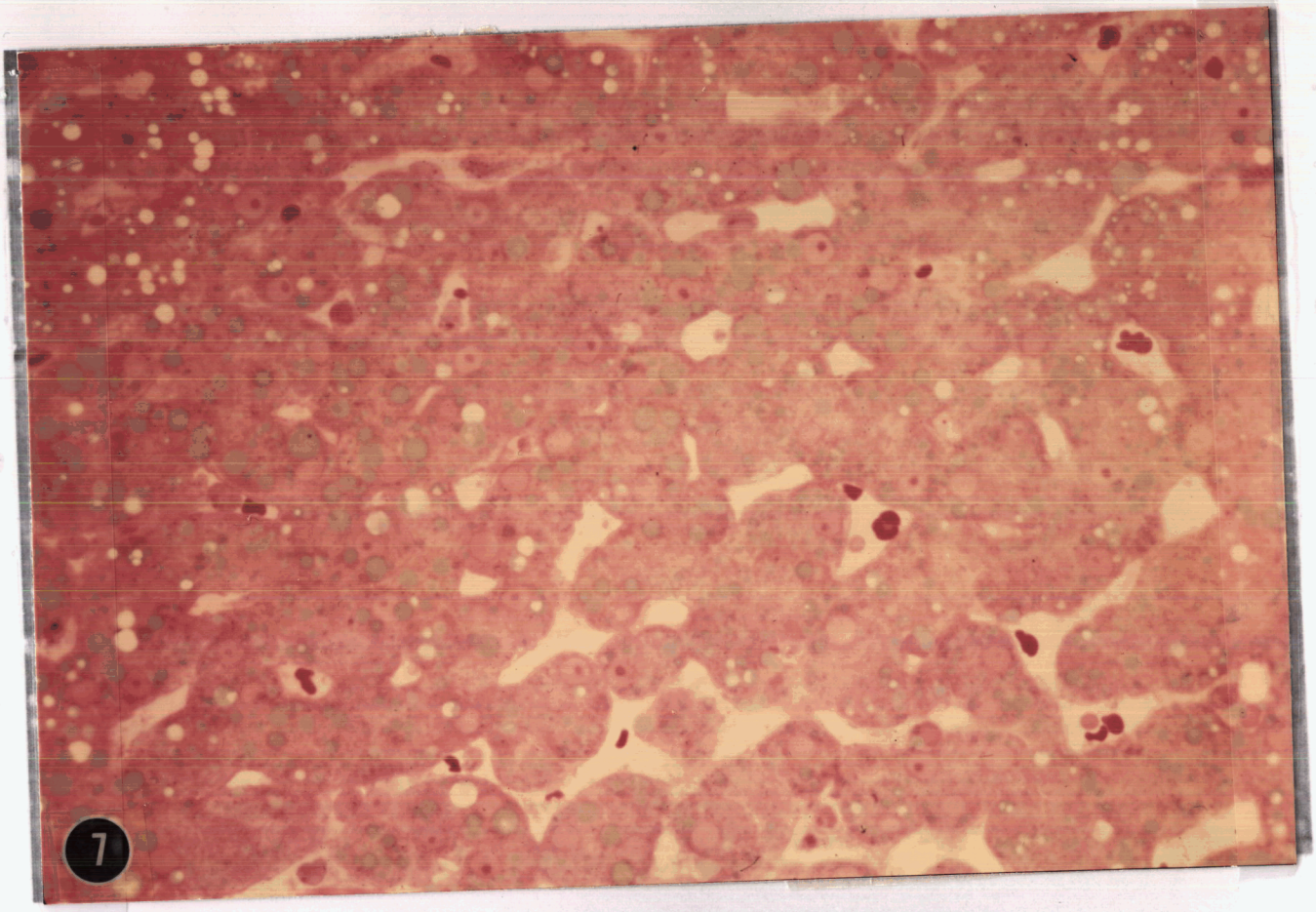

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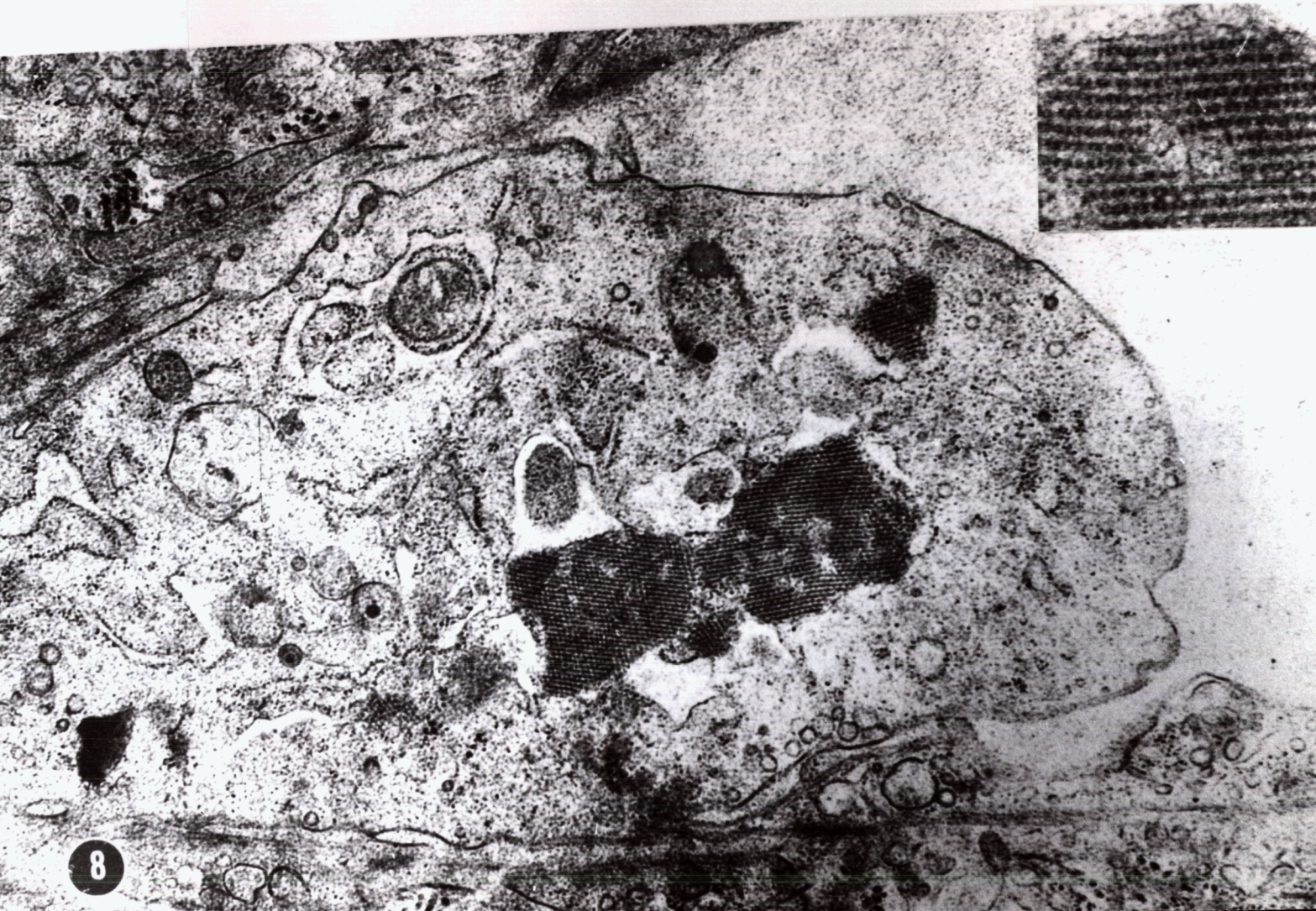

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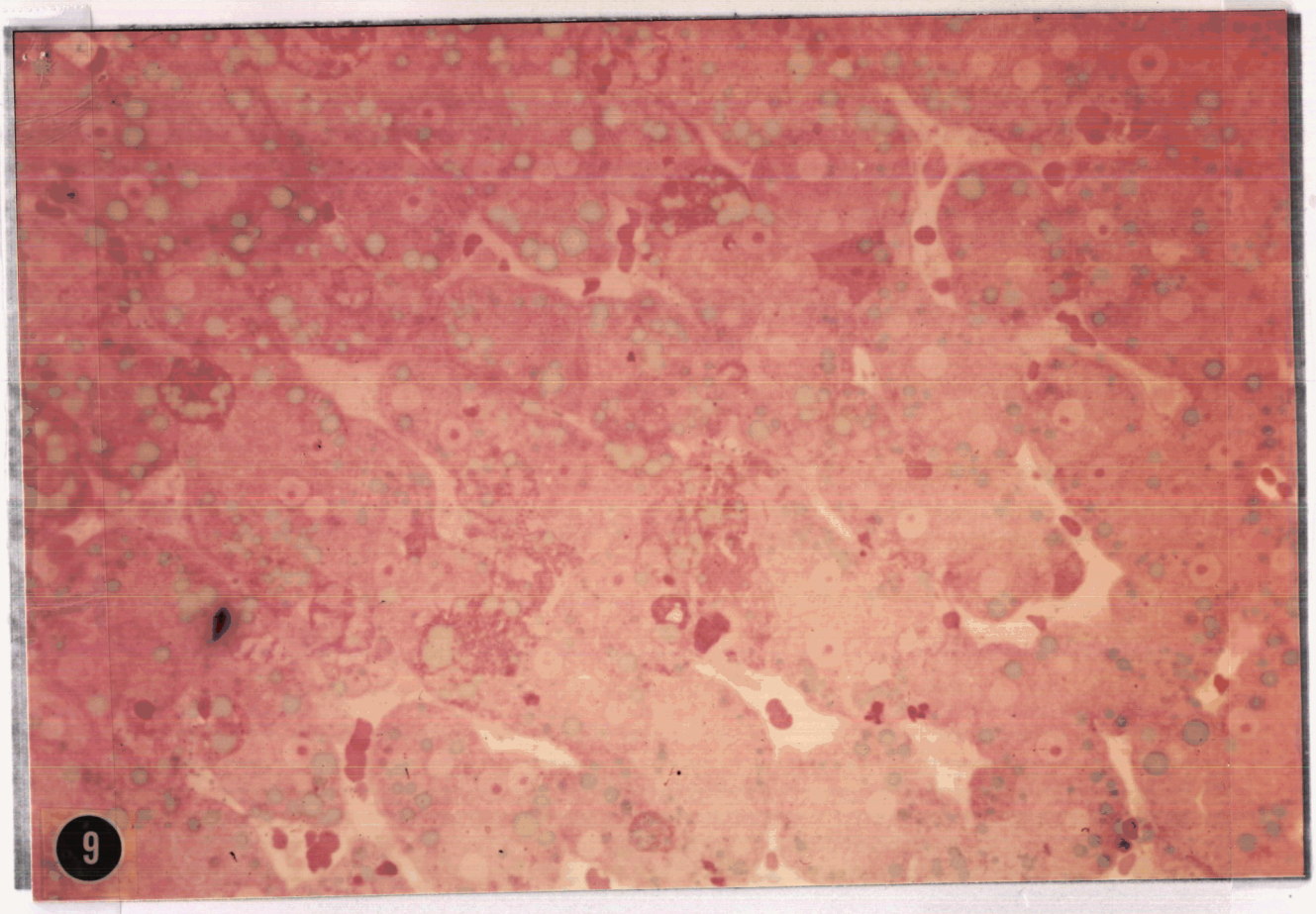

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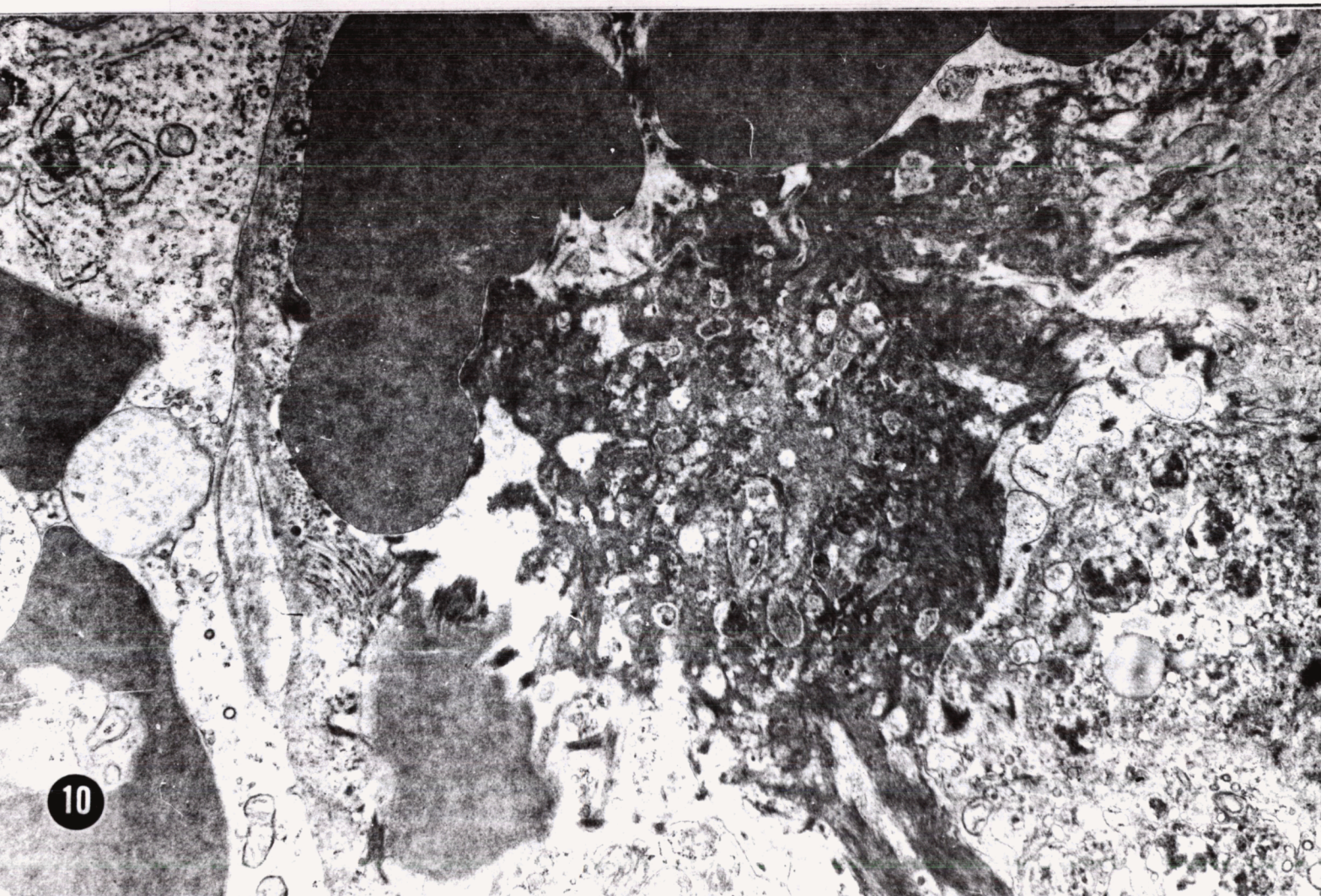

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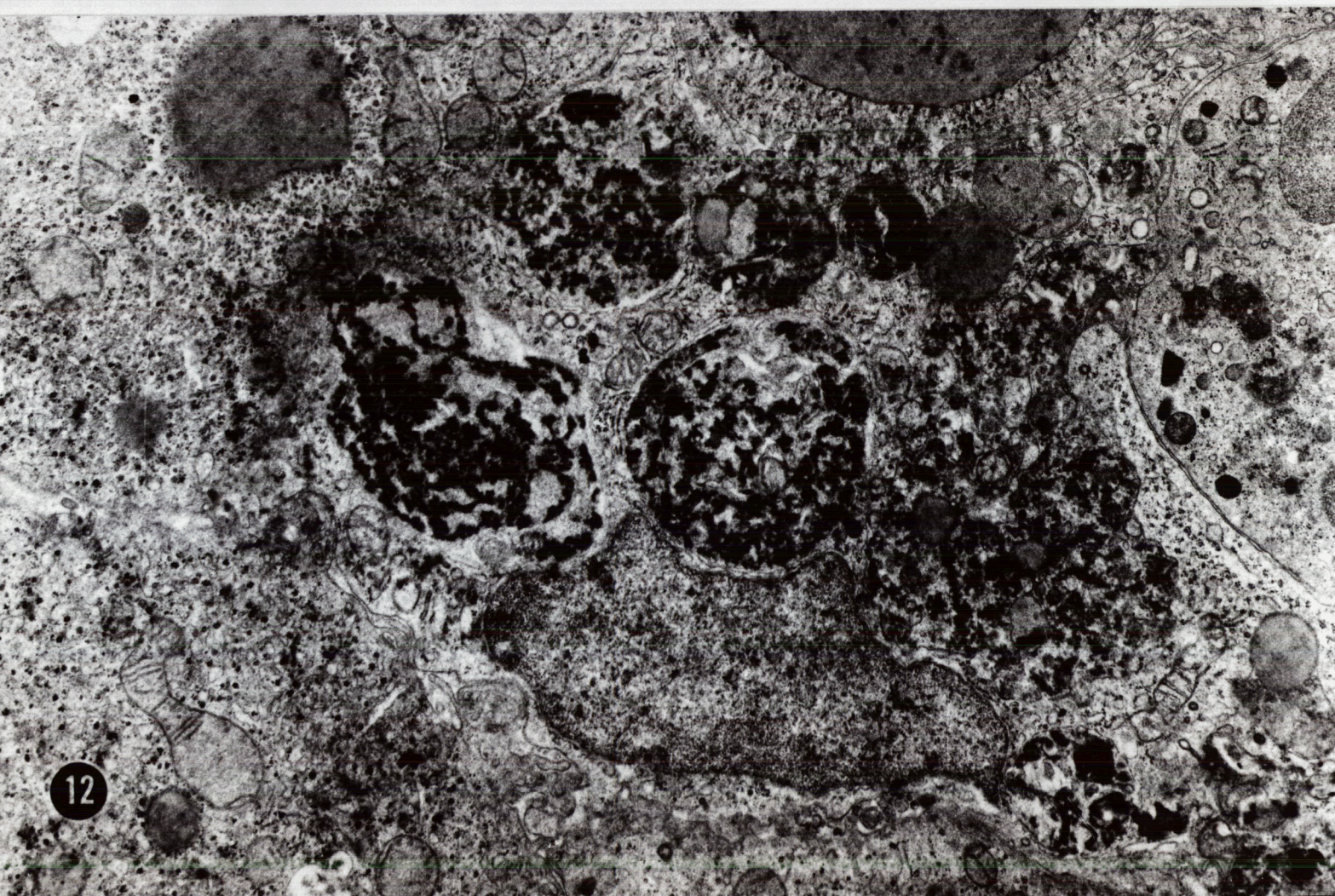

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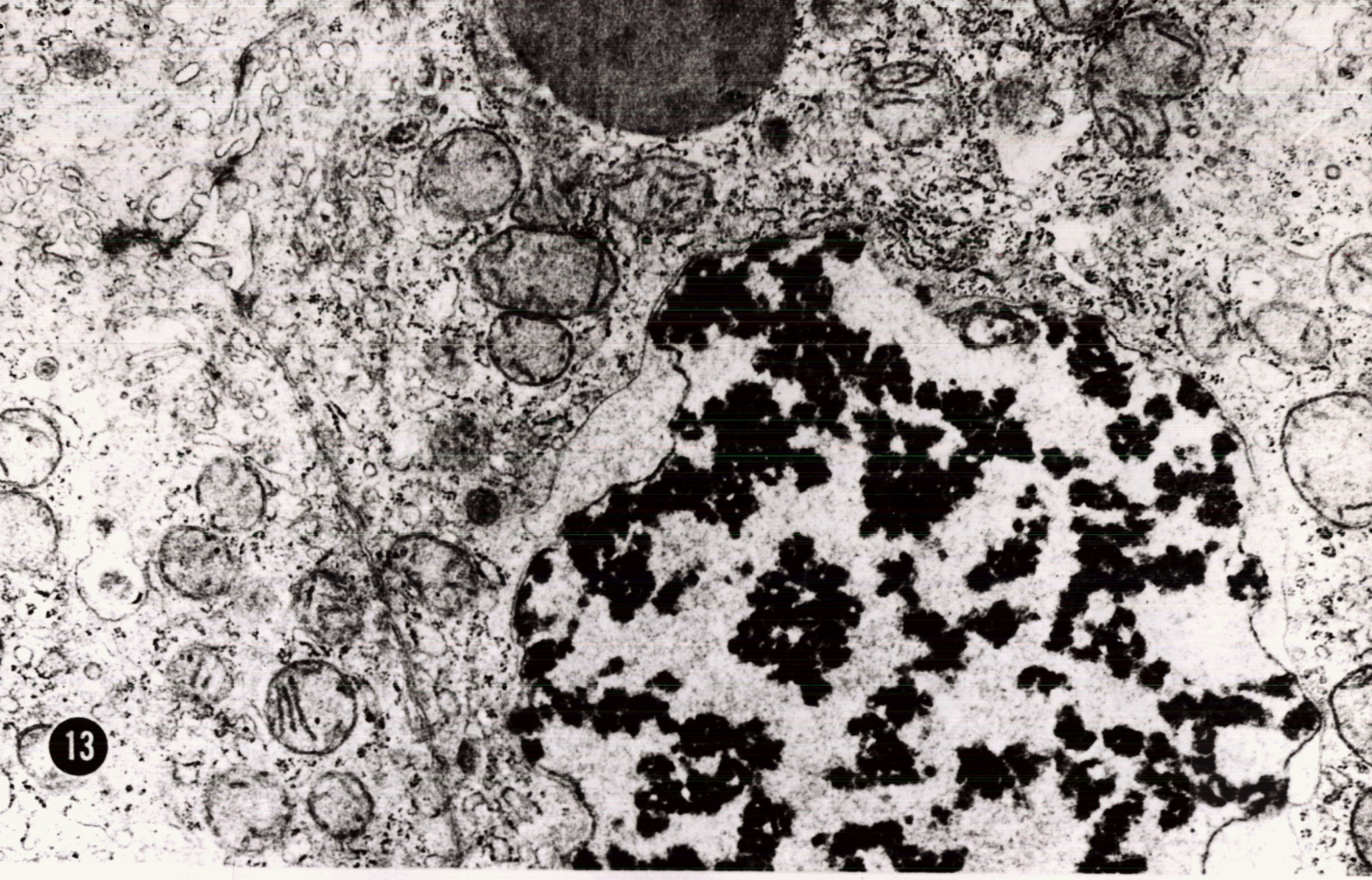

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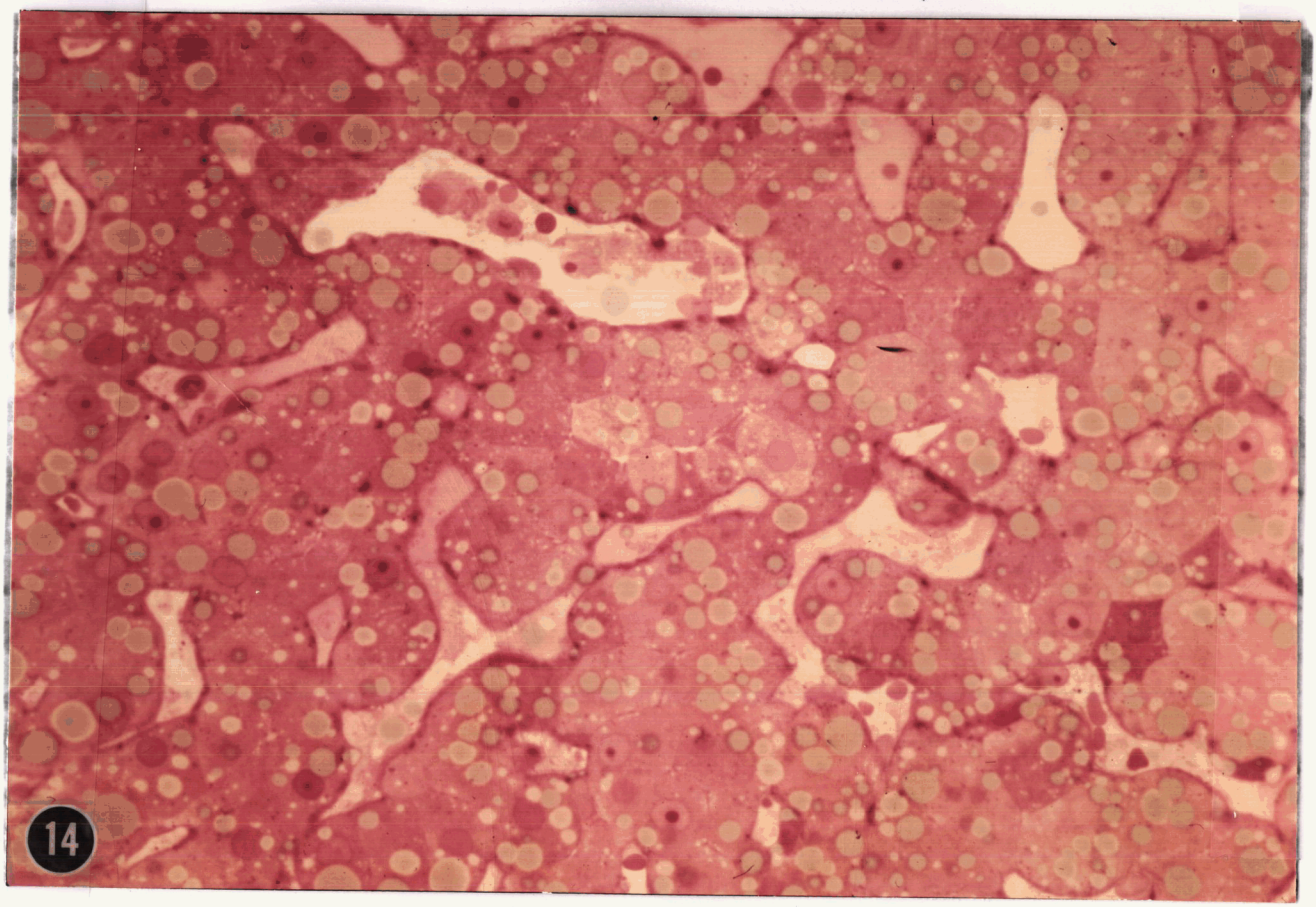

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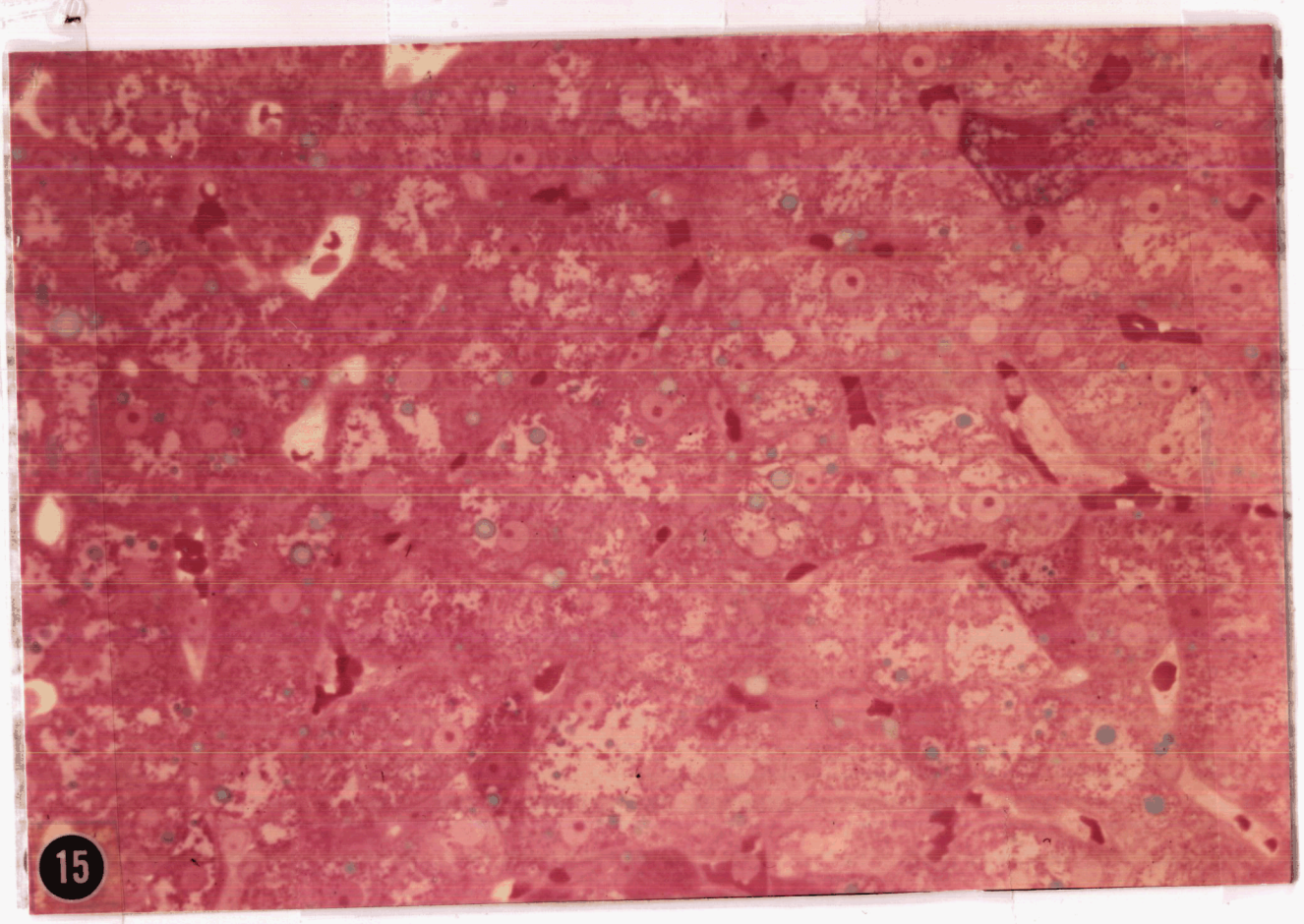
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